Human Dopamine (DA) ELISA Research Kit

Product code: 248623

Product Introduction

This kit is used to determine dopamine (DA) content in human serum, plasma, and related liquid samples.

The assay uses a double-antibody sandwich method. Purified human dopamine (DA) antibody is coated onto the microplate to form a solid-phase antibody. Dopamine (DA) in the sample binds to the coated antibody, and then binds with HRP-labeled dopamine (DA) antibody to form an antibody-antigen-enzyme-labeled antibody complex.

After thorough washing, TMB substrate is added for color development. Under HRP catalysis, TMB turns blue, and after the addition of acid it turns yellow. The color intensity is positively correlated with the dopamine (DA) concentration in the sample. Measure absorbance at 450 nm with a microplate reader and calculate the sample concentration from the standard curve.

Package Contents

Format	Item Code	Description	Quantity
48T	248623.1	20× Concentrated Wash Solution	20 mL × 1 bottle
48T	248623.2	Enzyme Conjugate Reagent	3 mL × 1 bottle
48T	248623.3	Sample Diluent	3 mL × 1 bottle
48T	248623.4	Chromogenic Reagent A Solution	3 mL × 1 bottle
48T	248623.5	Chromogenic Reagent B Solution	3 mL × 1 bottle
48T	248623.6	Stop Solution	3 mL × 1 bottle
48T	248623.7	Standard (240 ng/L)	0.5 mL × 1 vial
48T	248623.8	Standard Diluent	1.5 mL × 1 vial
48T	248623.9	Pre-coated Microplate	12 wells × 4 strips
48T	248623.10	Plate Sealer	2 sheets
48T	248623.11	Sealed Bag	1 pc
48T	248623.m	Instructions	1 copy

Quality Standards and Safety Instructions

Material and Packaging Name	Quality Standard	Main Toxicity
20× Concentrated Wash Solution	—	—
Enzyme Conjugate	—	—
Pre-coated Microplate	—	—
Sample Diluent	—	—
Color Reagent A Solution	—	—
Color Reagent B Solution	—	—
Stop Solution	—	—
Standard (240 ng/L)	—	—
Standard Diluent	—	—
Plate Sealer	—	—
Sealed Bag	—	—

Transportation and Storage

Transport with ice packs.

Store at 2-8°C. Shelf life: 180 days.

Instructions for Use

1. Standard Dilution

This kit provides one stock standard. Prepare standards in small test tubes as follows:

Standard	Target Concentration	Preparation
No. 5	120 ng/L	150 µL stock standard + 150 µL standard diluent
No. 4	60 ng/L	150 µL No. 5 standard + 150 µL standard diluent
No. 3	30 ng/L	150 µL No. 4 standard + 150 µL standard diluent
No. 2	15 ng/L	150 µL No. 3 standard + 150 µL standard diluent
No. 1	7.5 ng/L	150 µL No. 2 standard + 150 µL standard diluent

2. Assay Procedure

1. Set up blank wells, standard wells, and sample wells. Do not add sample or enzyme conjugate to the blank well. All other steps are the same.
2. Add 50 µL of each standard to the appropriate standard well.
3. For each sample well, add 40 µL sample diluent first, then add 10 µL sample for a final 5-fold dilution. Add the sample to the bottom of the well, avoid touching the wall, and gently shake to mix.
4. Seal the plate and incubate at 37°C for 30 minutes.
5. Dilute the 20× concentrated wash solution 20-fold with distilled water before use.
6. Remove the plate sealer carefully, discard the liquid, shake dry, fill each well with wash solution, let stand for 30 seconds, then discard. Repeat 5 times and pat dry.
7. Add 50 µL enzyme conjugate to each well except the blank well.
8. Seal and incubate again at 37°C for 30 minutes.
9. Wash again as described above.
10. Add 50 µL chromogenic reagent A to each well, then add 50 µL chromogenic reagent B. Gently shake to mix evenly.
11. Develop color at 37°C in the dark for 10 minutes.
12. Add 50 µL stop solution to each well. The blue color will immediately turn yellow.
13. Zero the instrument with the blank well and measure absorbance at 450 nm. Complete the reading within 15 minutes after adding the stop solution.

3. Result Calculation

Use the standard concentrations as the x-axis and OD values as the y-axis to draw a standard curve. Determine the corresponding sample concentration from the curve according to the sample OD value, then multiply by the dilution factor.

Alternatively, use the standard concentrations and OD values to calculate the linear regression equation for the standard curve. Substitute the sample OD value into the equation, then multiply by the dilution factor to obtain the actual sample concentration.

4. Summary of Assay Procedure

1. Prepare reagents, samples, and standards.
2. Add prepared samples and standards, then incubate at 37°C for 30 minutes.
3. Wash the plate 5 times, add enzyme conjugate, and incubate at 37°C for 30 minutes.
4. Wash the plate 5 times, add chromogenic reagents A and B, and develop color at 37°C for 10 minutes.
5. Add stop solution.
6. Read the OD value within 15 minutes.
7. Calculate the results.

Precautions

1. After removing the kit from refrigerated storage, allow it to equilibrate at room temperature for 15-30 minutes before use. If the enzyme-coated plate is not used up after opening, place the remaining strips in the sealed bag for storage.
2. The concentrated wash solution may form crystals. During dilution, warming in a water bath can help dissolve them and will not affect washing results.
3. Use a pipettor for each sample addition step, and check accuracy frequently to avoid experimental errors. One round of sample addition should preferably be completed within 5 minutes. If many specimens are tested, a multichannel pipette is recommended.
4. Prepare a standard curve for each assay, and it is best to run duplicate wells. If the analyte content is too high and the sample OD value is greater than the OD value of the first standard well, dilute the sample with sample diluent by n times before measurement. When calculating the result, multiply by the total dilution factor (n × 5).
5. The plate sealer is for one-time use only to avoid cross-contamination.
6. Store the substrate protected from light.
7. Follow the instructions strictly. Test results must be based on microplate reader readings.
8. All samples, wash solution, and wastes should be treated as infectious materials.
9. Do not mix components from different batch numbers.
10. Extract specimens as soon as possible after collection and perform extraction according to the relevant literature. Perform the assay as soon as possible after extraction. If immediate testing is not possible, samples may be stored at -20°C, but repeated freeze-thaw cycles should be avoided. Samples containing NaN3 cannot be tested because NaN3 inhibits horseradish peroxidase (HRP) activity.
11. Detection range: 5 ng/L-120 ng/L.

Visual Reference